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NITROGEN BACTERIA AND LEGUMES

(WITH SPECIAL REFERENCE TO RED CLOVER, COWPEAS, SOY BEANS, ALFALFA, AND SWEET CLOVER, ON ILLINOIS SOILS).

By CYRIL G. HOPKINS.

Work and Knowledge are a stronger team than Work and Work.

URBANA, ILLINOIS, FEBRUARY, 1904.

Summary of Bulletin No. 94.

1. Soil nitrogen cannot be used by plants until it is changed to the form of nitrate nitrogen by the nitrifying bacteria. Page 307

2. Atmospheric nitrogen cannot be used by any agricultural plants, excepting legumes, and even leguminous plants have no power to obtain nitrogen from the air unless they are provided with the proper nitrogen-gathering bacteria. Page 309

3. As a rule each important agricultural legume must have its own particular species of bacteria. Page 311

4. The frequent failure of red clover in normal seasons, especially on normal soils occupying the highest land, is undoubtedly due in part at least to the absence of the proper bacteria (sometimes the soil lacks lime or phosphorus). Page 313

5. On the very acid soils, where clover has never been grown successfully, applications of ground limestone should be made where legumes are to be grown. Page 315

6. Cowpeas need not be inoculated, because the cowpea bacteria are usually either present in the soil or are introduced with the seed in sufficient numbers to effect a good degree of infection if the soil is suitable and if cowpeas are seeded upon the same land for two successive years. Page 317

7. Cowpeas grown on infected soil on the University of Illinois soil experiment field contained four times as much nitrogen as the same kind of cowpeas grown on similar land which was not infected. Page 319

8. As a rule soy bean fields should be inoculated when first seeded to soy beans, otherwise they may be grown on the same land for three or four years before the soil becomes thoroughly infected. Page 320

9. Investigations, reported in this bulletin, furnish conclusive proof that infected sweet clover soil can be used for inoculating alfalfa fields and with the same results as are obtained when the infected soil is obtained from an old alfalfa field. (Sweet clover is a tall, rank-growing, sweet-scented leguminous plant widely distributed over Illinois, especially along the roadsides in the northern and central parts of the state, and in many places in bottom lands in southern Illinois.) Page 324

10. This bulletin will be sent free of charge to any one interested in Illinois agriculture upon request to E. Davenport, Director Agricultural Experiment Station, Urbana, Illinois; and, if so requested, the name of the applicant will be placed upon the permanent mailing list of the Experiment Station, so that all bulletins will be sent to him as they are issued.

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NITROGEN BACTERIA AND LEGUMES

(With Special Reference to Red Clover, Cowpeas, Soy Beans, Alfalfa, and Sweet Clover, on Illinois Soils).

By CYRIL G. HOPKINS, Chief in Agronomy and Chemistry.

Among the several different classes or groups of bacteria there are two which are of special importance to agriculture because of their relation to the element nitrogen, this being commonly considered the most valuable element of plant food.* These two classes of bacteria are, first, the nitrifying bacteria, and, second, the nitrogen-gathering bacteria.

The Nitrifying Bacteria.

The nitrifying bacteria are those which have the power to form nitrates. In the following brief discussion of this subject we include at least three species of bacteria which by their combined or successive action have the power to transform organic nitrogen into nitrate nitrogen, which is a suitable form of nitrogen for plant food. For the exact information which we now have regarding the nitrifying bacteria we are indebted to the researches of Pasteur and Schlösing and Müntz of France, Winogradsky of Russia, Warington of England, and others.

The nitrogen in the soil is almost entirely in organic compounds; that is, the nitrogen (which is a gas in the free, or uncombined, state) is united or combined with other elements, notably with carbon, hydrogen, and oxygen, in the form of partially decayed vegetable or organic matter. (By organic matter we mean matter which has been formed by the growth of some organism, either plant or animal, as grass or flesh.) Plants cannot use the free nitrogen of the air as plant food, neither can they use the organic compounds of nitrogen which occur in the soil. There

*It should be remembered that there are ten essential elements of plant food each of which is of equal importance to the plant, for if the plant is deprived of any one of the ten essential elements it is impossible for it to develop and mature. Carbon has no market value as plant food because the plant obtains carbon in the form of of carbon dioxid, a gas which is present everywhere in the atmosphere and which the plant inhales through its leaves. Both hydrogen and oxygen are without market value because they are the elements which compose water, a liquid compound which plants absorb through their roots. Calcium, magnesium, iron, and sulfur have no market value as elements of plant food because they are present in practically all soils in abundance as compared with the amounts required in plant growth. The three elements nitrogen, phosphorus, and potassium, do have market values, because they are required by plants in very considerable quantities, and they are present in most soils in rather limited amounts, and when the available supply of any one of these elements becomes too much reduced in a soil the crop yield also becomes reduced. For further information regarding the use of these elements of plant food on Illinois soils, see Circular No. 68, "Methods of Maintaining the Productive Capacity of Illinois Soils."

are at least three different kinds of bacteria, and also three different steps or stages involved in the process of nitrification, the nitrogen being changed from the organic compounds first into the ammonia* form, second, into the nitrite form, and third into the nitrate form. During the process the nitrogen is separated from the carbon and other elements composing the insoluble organic matter, and is united or combined with oxygen and some alkaline element (as calcium) to form the soluble nitrate, such as calcium nitrate, which is one of the most suitable compounds of nitrogen for plant food. Calcium is the alkaline element contained in lime or limestone. The name *calcium nitrate* indicates just what elements this compound contains; namely, calcium, nitrogen, and oxygen. (In the names of compounds the ending *-ate* always means oxygen.)

This is the general process of nitrification in which the nitrifying bacteria transform or transfer the nitrogen from insoluble organic compounds into soluble compounds in which it may serve as available plant food. The nitrate which is thus formed may be calcium nitrate or magnesium nitrate or potassium nitrate or even sodium nitrate, depending upon which of these alkaline elements is present in the must suitable form. If no alkaline element is present in available form then no nitrates can be made in the soil. One of the reasons for applying ground limestone to soils which are deficient in lime is to furnish the element calcium in suitable form for the formation of nitrates in the process of nitrification. Ground limestone is calcium carbonate ($CaCO_3$), a compound containing one atom of calcium (Ca), one atom of carbon (C) and three atoms of oxygen (O_3). This is the same form of lime which is contained naturally in limestone soils—soils which are noted for their great productiveness—and it is generally the most economical form of lime to use for correcting soil acidity and promoting nitrification.

In the process of nitrification, that is in the formation of nitrates, there is required, not only the presence of calcium, or some other alkaline element, in suitable form, but also a good supply of the element oxygen; for calcium nitrate, $Ca(NO_3)_2$, contains one atom of calcium (Ca), two atoms of nitrogen $(N)_2$, and six atoms of oxygen $(O_3)_2$, in each molecule as indicated in the formula, $Ca(NO_3)_2$. Magnesium nitrate, $Mg(NO_3)_2$, potassium nitrate, KNO_3 (K is from the Latin word *Kalium*, which means potassium), and all other nitrates, also, contain oxygen. The supply of oxygen for the formation of nitrates in the soil comes from the air, which consists of about twenty percent oxygen, seventy-eight percent nitrogen, and two percent of other elements and compounds, as argon, carbon dioxid, CO_2, water vapor, H_2O, etc. One of the important effects of cultivation, or tillage, is that it permits the air more freely to enter the soil, and thus promotes nitrification.

*Technically this first step is preliminary to, and not a part of, nitrification.

THE NITROGEN-GATHERING BACTERIA.

As stated above, the nitrogen naturally in the soil is contained almost entirely in the organic matter. Any process which tends to decompose or destroy this organic matter, such as nitrification or other forms of oxidation, will also tend to reduce the total stock of nitrogen in the soil. Because of this fact the matter of restoring nitrogen to the soil becomes of very great importance. Of course a part of the nitrogen removed in crops may be returned in the manure produced on the farm; and nitrogen may also be bought in the markets in such forms as sodium nitrate (containing 15 to 16 percent of nitrogen), ammonium sulfate (containing 20 to 21 percent of nitrogen), and dried blood (containing 12 to 15 percent nitrogen); but, when we bear in mind that such commercial nitrogen costs about 15 cents a pound, and that one bushel of corn contains about one pound of nitrogen, it will be seen at once that the purchase of nitrogen cannot be considered practicable in general farming, although in market gardening, and in some other kinds of intensive agriculture, commercial nitrogen can often be used with very marked profit.

Nitrogen is removed from the soil not only in the crops grown, but also, and frequently in larger amounts per annum, in the drainage waters, and in some other ways, as by denitrification and by the blowing and washing of the surface soil. Professor Snyder, of the Minnesota Experiment Station, has shown that during a series of years the total loss of nitrogen from some Minnesota soils in some cases amounts to several times the amount actually used in the crops produced.

Considering all of these facts, and the additional facts that there are about seventy-five million pounds of atmospheric nitrogen resting upon every acre of land, and that it is possible to obtain unlimited quantities of nitrogen from the air for use of farm crops, and at very small cost, the inevitable conclusion is that the inexhaustible supply of nitrogen in the air is the store from which we must draw to maintain a sufficient amount of this element in the soil for the most profitable crop yields.

It is often stated that leguminous plants, such as clover, have power to obtain free nitrogen from the air. This is not strictly true. Red clover, for example, has no power in itself to get nitrogen from the air. It is true, however, that the microscopic organisms* which commonly live in tubercles upon the roots of the clover plant do have the power to take free nitrogen from the air and cause it to unite with other elements to form compounds suitable for plant food. The clover plant then draws

*Among the scientists who were prominent in making these discoveries regarding the action of bacteria in the fixation of atmospheric nitrogen were Hellriegel, Willfarth, and Nobbe in Germany, Atwater in America, Lawes and Gilbert in England, and Boussingault and Ville in France.

upon this combined nitrogen in the root tubercles, and makes use of it in its own growth, both in the tops and in the roots of the plant.

These nitrogen-gathering bacteria live in tubercles upon the roots of various leguminous plants,* such as red clover, white clover, alfalfa, sweet clover, cowpeas, soy beans, vetch, field-peas, garden-peas, field and garden beans, etc. These tubercles vary in size from a pinhead to a pea, varying with the different kinds of plants, being especially small upon some of the clovers, and very large upon cowpeas and soy beans.

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*It may be well to call attention to the fact that there are numerous instances where two different kinds of plants live together in intimate partnership relation. If only one of the two plants receives benefit from this relationship or association, then the plant receiving the benefit is called a parasite. Thus the mistletoe is a parasite upon the elm or gum or other tree on which it lives. The mistletoe draws its nourishment from the tree. The tree is injured rather than benefited by the mistletoe. Dodder is also a parasitic plant, living upon other plants, except during the early part of its growth. Ticks and lice are common examples of animal parasites living upon other animals.

In some cases a relationship exists which is not parasitic but symbiotic. The term symbiosis, which is commonly used by biologists to define this relationship, means living together in mutual helpfulness. The association of bees and flowers may serve to illustrate this mutual helpfulness, although this is not an example of intimate symbiosis. Thus the bees obtain their food from the flowers and, in turn, the flowers, many of them, are incapable of producing seed or fruit unless the pollen is carried from the male flower to the female flower by bees or other agencies. It is well known that plant lice and ants are mutually helpful.

Likewise the association of nitrogen-gathering bacteria and leguminous plants is a relationship of mutual helpfulness and this is one of the best illustrations of what is meant by symbiosis. The legume furnishes a home for the bacteria and also furnishes in its juice or sap most of the nourishment upon which the bacteria live. The bacteria, on the other hand, take nitrogen from the air contained in the pores of the soil, and cause this nitrogen to combine with other elements in suitable form for plant food which is then given up to the legume for its own nourishment.

Another illustration of remarkable parasitism, if not, indeed, one of true symbiosis, is found in the common lichens living upon rocks and trees. The lichen is not a single plant, but two plants—an alga, which lives upon the wood or stone, and a fungus which lives upon the alga. Algæ also live in the free state separate from fungi, and the present opinion of botanists seems to be that when the two are associated in the form of lichens this association is not detrimental, but rather beneficial, to the alga, as well as to the parasitic fungus. If this is true, then it is another case of true symbiosis. (There is reason to believe that the fungus has some power to feed upon atmospheric nitrogen, and then to furnish combined nitrogen to the alga upon which it lives.)

In the symbiosis of leguminous plants and nitrogen-gathering bacteria we have a partnership or relationship of immeasurable value to agriculture. Here is a class of plants (legumes) that are capable of consuming or utilizing nitrogen in quantities larger than could possibly be obtained from ordinary soils for any considerable length of time. They have no power in themselves of taking nitrogen from the atmosphere, and to them the symbiotic relation with this low order of plants (the nitrogen-gathering bacteria) is especially helpful, and for the best results it is absolutely necessary.

The tubercles are, of course, easily seen with the eye, but the tubercle is only the home of the bacteria, somewhat as the ball upon the willow twig is the home of the insects within. The bacteria themselves are far too small to be seen with the unaided eye, although they can be seen by means of the most powerful microscope. Several million bacteria may inhabit a single tubercle. It is not necessary to see the bacteria, because if we find the tubercles upon the roots of the plant, we know that the bacteria are present within, as otherwise the tubercle would not be formed.

Although the plant itself, as clover, for example, has no power to feed upon the free or uncombined nitrogen in the air, yet these nitrogen-gathering bacteria do have the power to absorb the free nitrogen and cause it to combine with other elements, forming nitrates or other compounds which are suitable forms of nitrogen for plant food.

It has also been demonstrated that, as a rule, there are different species of nitrogen-gathering bacteria for markedly different species of leguminous plants. Thus we have one kind of bacteria for red clover, another kind for cowpeas, another kind for soy beans, and still a different kind for alfalfa.*

THE RED CLOVER BACTERIA.

That clover has no power in itself to gather atmospheric nitrogen, and that the bacteria do have power to feed the clover plant with nitrogen gathered from the air is very easy to demonstrate. It is one of the regular laboratory practices of the students in soil fertility in the Agricultural College to make this demonstration. Plate 1 is an illustration of such student work. The two pots which are shown were provided with all elements of plant food, excepting the one element nitrogen. Thus far the two pots are exactly alike. Each contains no nitrogen, as indicated by the label "No N." Each pot is planted with the same number of red clover seeds. To the right-hand pot, however, some bacteria ("Bac.") were added, while none were added to the left-hand pot. These bacteria were obtained by taking about one pound of soil from a clover-field where abundance of tubercles were found on the

*There are some noteworthy exceptions to this rule (see following pages for illustration), and there is some evidence that, by a comparatively long process of breeding, or evolution, the bacteria which naturally live upon one kind of legume may gradually develop the power to live upon a distinctly different legume to which they were not at first adapted. Of course this process of forcing bacteria to live upon a legume to which they are not naturally adapted has little or no practical value because it is unnecessary if there is a species of bacteria which naturally lives upon the same legume. On the other hand, if, by any such process of breeding, or evolution, a species of nitrogen-gathering bacteria could be developed which could live on a non-leguminous plant, as corn, for example, it would be of incalculable practical value. As yet the efforts of bacteriologists, working on this problem, have given only negative results, so far as known to the writer.

PLATE 1. RED CLOVER: EFFECT OF BACTERIA. NO NITROGEN IN THE SOIL OF EITHER POT.

clover roots, adding this soil to about one quart of pure water, shaking for a few minutes, allowing the soil to settle, then taking a small quantity of almost clear solution, and adding it to the pot which we wished to inoculate with the red clover bacteria. Aside from the addition of these microscopic bacteria to the right-hand pot, these two pots were treated exactly alike throughout the experiment. It will be plainly seen that where the bacteria were added the clover was furnished with sufficient nitrogen to make a strong and luxuriant growth, while without the bacteria the clover (in the left-hand pot) only germinated and made what little growth it could with the small amount of nitrogen contained in the seed. This result is the difference between success and failure of the clover crop.

In general the clover bacteria are well distributed over the northern and central part of Illinois, but we now have some very strong evidence that they are not well distributed in some soils of large area in southern Illinois. There is also some evidence that they were not originally

present even in the soils where they are now found in great abundance; and, furthermore, it seems very probable that these bacteria may cease to live in a soil where they have once been present, provided clover is not grown on the land for several years.

It will help us to understand this matter if we bear in mind that the home of these bacteria is the tubercle upon the clover root. It is quite evident that they will continue to live upon the decaying tubercles or roots for three or four years after the clover plant has been killed. On the other hand, we have some notable evidence that the bacteria do not continue to live in a soil after five or six years' continuous cropping with absolutely no clover growing on the land during those years. It is a simple matter for any one to determine whether the bacteria are present or not, for the tubercles which are formed if the bacteria are present are plainly seen attached to small roots. They look somewhat like miniature potatoes, varying in size from pinheads on clover to peas on soy beans or cowpeas. (See Plates 2 and 4.) It is important to remember that the bacteria live in the soil and not in the seed.

When clover is cut for seed, it is frequently left to lie upon the ground until the straw becomes half rotten and very dirty; and, consequently when it is threshed, it practically always happens that there is at least some small amount of dust and dirt taken with the seed. This dirt is almost sure to carry with it some bacteria from the soil. If these few bacteria are scattered with the clover seed when it is sowed they will inoculate at least a few plants, and if they are allowed to multiply on these plants, and especially if the same field is repeatedly seeded with clover, the soil will ultimately become thoroughly infected with the clover bacteria. Of course they may be carried from one part of the farm to another, or even from one farm to another, by various agencies, as dust or wind storms, surface drainage or flood waters, manure made from clover hay, implements used in cultivating the soil, etc., etc.

Many of the older farmers of Illinois have stated to the writer that when this country was very new it was commonly found difficult to get a "catch" of clover on new land. After a good "catch" was once gotten, then it was easier to get clover to grow on that land the next time. There was a saying among the farmers that clover would not do well until they got the "wild nature" out of the land. Their final success was undoubtedly due, not to getting anything out of the land, but rather to getting the bacteria into the land. Several Illinois farmers have reported some quite remarkable results from very light applications of the clover chaff or straw (obtained in hulling clover) in its beneficial effect on clover on land where it was otherwise difficult to get a "catch." There is a somewhat general belief among farmers of long experience that clover straw or chaff has some special value in getting a catch of clover aside from its value as manure or for the seed which it sometimes contains.

Manager F. A. Warner of the Sibley Estate, Ford County, recently stated to the writer that they had had very great difficulty to get clover to grow when they first began growing clover on that large estate, some six or eight years ago, although, after a good crop was once secured, they rarely had any further difficulty in getting a catch of clover on the same land.

On the common gray prairie soil of the Lower Illinoisan Glaciation, in southern Illinois, the commonest type of soil in more than twenty counties, practically no red clover is grown. In the spring of 1903 we seeded red clover on that type of soil in three places; namely, on the University of Illinois soil experiment fields near Edgewood, Effingham County, near Du Bois, Washington County, and near Cutler, Perry County. On certain plots the soil acidity had been corrected with lime and an abundant supply of phosphorus (in bone meal) had been provided, potassium also having been added on some plots. These fields were carefully examined the latter part of June, and at Cutler and Du Bois the clover was found to be dead or dying, and no tubercles could be found upon the clover roots, although on the clover which had been seeded at about the same time on the University fields at Urbana the root tubercles were found in great abundance. At Edgewood a few tubercles were found and the clover appeared to be growing fairly well. Infected red clover soil was at once procured and scattered over the fields at Edgewood, Du Bois, and Cutler, but it was evidently too late to be of any marked benefit. At Cutler and at Du Bois the clover was a complete failure. (It will be tried again next year.) At Edgewood it continued to grow fairly well, and its progress next season (1904) will be watched with much interest. It should be stated that the Experiment Station has been growing clover for several years with varying degrees of failure on land adjoining the present clover field at Edgewood, and it is possible that this year's apparent success from the start is due in part at least to the bacteria which have been incidentally introduced and multiplied year after year and scattered over the adjoining land by wind and dust storms. Before the close of the season the tubercles developed in abundance on the roots of the clover at Edgewood.

An experience reported by Professor Herbert W. Mumford, of the Animal Husbandry Department of this university, will be of interest and value in this connection. Professor Mumford commonly grows clover in his rotations on his own private farm, but he states that at one time one particular field was cropped continuously with timothy, oats, and corn for some six years or more without any clover whatever. It was then again seeded to clover, but the crop made a complete failure, although on other land where clover had been grown more recently a successful clover crop was grown from the same kind of seed seeded at about

the same time. The following year this particular field was again seeded to clover. This time the "catch" was not a total failure, but it was too poor to save, and it was plowed up and the land again seeded to clover the next year, and an excellent catch of clover resulted. After this, clover was frequently grown on this field, and no special difficulty was had in getting good crops.

While the failure of clover may often be due to drouth, and in some places due to soil acidity (lack of lime), and sometimes even due to an insufficient supply of available phosphorus or of potassium, we now know with certainty that it sometimes fails because of the absence of the nitrogen-gathering bacteria, especially on land which has never grown clover, and probably also on land which has not grown it recently. We should always remember that the bacteria do not thrive in strongly acid soils. Even though they may sometimes live in such soils and perhaps produce some tubercles upon the roots of certain hardy, strong growing legumes, like cowpeas, nevertheless we are obtaining some strong evidence that in such acid soils they have but little power to gather nitrogen from the air. That ground limestone is the most economical and satisfactory material to use in correcting the acidity of soils is strongly indicated by the information we have thus far obtained. On the upland prairie soils of the Lower Illinoisan Glaciation where red clover has never been grown successfully, largely because of the acidity of the soil, it will undoubtedly be helpful and profitable not only to correct the acidity of the soil with ground limestone, but also to secure infected soil from some field of timber land or bottom land where red clover is growing, well provided with root tubercles, and inoculate the field with it. This soil should be collected to a depth of three or four inches and scattered over the prairie land at the rate of a few hundred pounds per acre at the time the clover is seeded or before.

THE COWPEA BACTERIA.

Plate 2 is made from a photograph of a cowpea root with the tubercles upon it. This illustration shows the cowpea tubercles at nearly natural size, which is about as large as the seed of ordinary garden peas.

The cowpea bacteria are already quite widely distributed in southern Illinois, especially where this crop has been grown for several years, but they are not common in the soils of other parts of the state. It is doubtful, however, if it is necessary or even worth while to take the trouble to inoculate soil for cowpeas. Some few tubercles almost invariably develop on cowpea roots the first year they are seeded, even where they have never been grown before, and if seeded the second year on the same land the plants are usually abundantly provided with root tubercles.

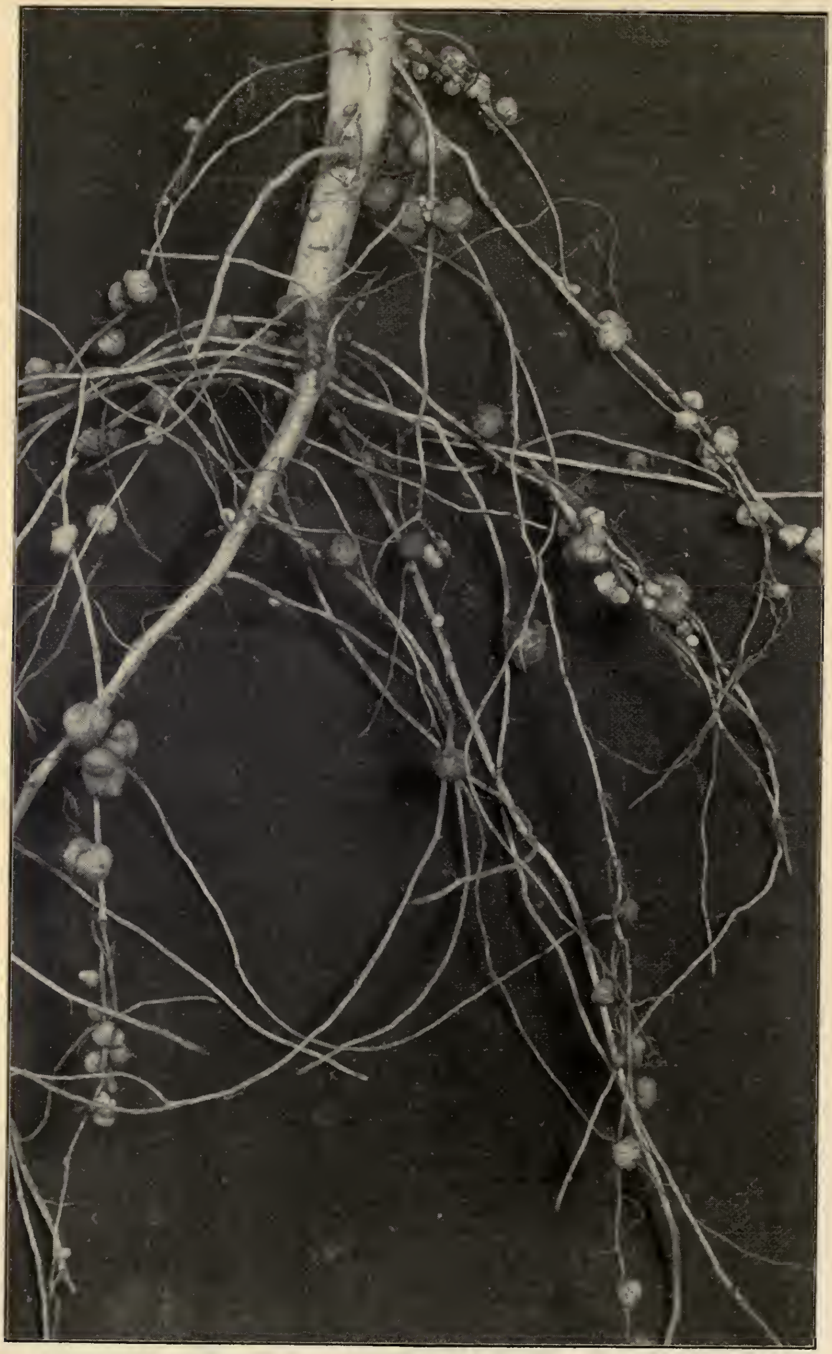

PLATE 2. COWPEA ROOT TUBERCLES, NATURAL SIZE.

Just why the cowpea bacteria develop so rapidly even without special inoculation is not definitely known. It may be that the same bacteria also live on some other leguminous plant which is more or less widely distributed over the state, but it seems more likely that the bacteria are brought with the seed. As a matter of fact, the cowpea harvest is usually dirty. This is an annual plant, and consequently the crop is grown on recently plowed land and is sometimes cultivated during the season. Cowpeas are commonly harvested with a mowing machine and then raked up on the loose ground. When they are threshed more or less dirt remains with the seed. Furthermore, the seed coats are not infrequently cracked, thus providing an excellent place for the lodgment of particles of soil.

Whether it would be profitable to inoculate the land for cowpeas would depend very largely upon the difficulty or cost of obtaining the infected material. If soil thoroughly infected with the cowpea bacteria can be scattered over the land at the rate of about 2,000 pounds to the acre at a cost of $1.00 or less per ton, it might prove profitable. It is doubtful if a light application of 100 or 200 pounds would produce any very marked effect in the yield the first season. After the soil becomes well infected the cowpeas then obtain much nitrogen from the air, and the yield of cowpeas is likely to be largely increased. Of course there is no fixation of atmospheric nitrogen if there are no tubercles on the roots.

In 1902 several plots of cowpeas were seeded on the soil experiment field at the university. One of these plots (404) had become thoroughly infected with the cowpea bacteria because of its being so situated that more or less surface drainage water flowed over it from an adjacent field upon which cowpeas had been grown for three successive years. Another plot (408), owing to a slightly different situation, had not become infected. The two plots were seeded in July after a crop of oats had been removed from the land. Within three weeks after seeding, numerous root tubercles could be found on the plants on the infected plot. Later on, ten average consecutive plants were taken up as completely as possible, and 412 tubercles were found on the roots, making an average of more than 40 tubercles to the plant. On Plot 408 only an occasional plant was found infected, and such plants would usually have only a single large tubercle on their roots. Ten average plants not infected were collected from Plot 408 for comparison with the ten infected plants from Plot 404.

PLATE 3. COWPEAS: EFFECT OF BACTERIA IN ORDINARY ILLINOIS BLACK PRAIRIE SOIL.

Plate 3 shows these two bunches of plants, the infected plants with root tubercles on the *right*, and the plants without tubercles on the *left*. Four more sets of ten plants each were then collected, two sets from Plot 404 and two from Plot 408. Each set of infected plants was separated into three parts, (1) tops, (2) roots, (3) tubercles; and each set of plants not infected was separated into (1) tops, and (2) roots. All of these samples were dried and analyzed for nitrogen. The results obtained are shown in Table I.

The results clearly show the very great value of the nitrogen-gathering bacteria in growing cowpeas. In each of the separate trials A, B, and C, the infected plants contained about twice as much total dry matter as the plants not infected. The infected plants also contained a much higher percent of nitrogen than the plants not infected, the infected plants containing 4.09 to 4.33 percent in the tops and 1.45 to 1.53 percent in the roots, while those not infected contained only 2.32

TABLE I.—FIXATION OF NITROGEN BY COWPEAS.

Cowpea Plants. No.	Part.	Dry matter, cgs.	Nitrogen, content, percent.	Nitrogen, amount, cgs.	Nitrogen fixed by bacteria, cgs.
A1—Ten plants, with bacteria present	Tops	3580	4.09	146	
	Roots	620	1.45	9	
	Tubercles	190	5.97	11	
	Total	4390		166	125
A2—Ten plants, without bacteria	Tops	1560	2.42	38	
	Roots	300	.88	3	
	Total	1860		41	
B1—Ten plants, with bacteria present	Tops	3970	4.31	171	
	Roots	690	1.47	10	
	Tubercles	300	6.05	18	
	Total	4960		199	140
B2—Ten plants, without bacteria	Tops	2060	2.69	55	
	Roots	430	.88	4	
	Total	2490		59	
C1—Ten plants, with bacteria present	Tops	3300	4.33	143	
	Roots	520	1.53	8	
	Tubercles	290	5.76	17	
	Total	4110		168	124
C2—Ten plants, without bacteria	Tops	1730	2.32	40	
	Roots	400	.88	4	
	Total	2130		44	

to 2.69 percent in the tops and .88 percent in the roots. Besides this, the tubercles on the infected plants contain 5.76 to 6.05 percent of nitrogen. In these young and rapidly growing plants the tubercles are much richer in nitrogen than any other part of the plant. It should be stated that as the plants approach maturity the nitrogen is largely absorbed from the tubercles and stored in the tops and roots. At the time these plants were taken up the tubercles actually contained more nitrogen than the roots. The infected plants contained nearly four times as much nitrogen as the plants not infected, and about three-fourths of the total nitrogen in the infected plants was obtained from the air. The roots and tubercles of the infected plants contained six to seven times as much nitrogen as the roots of the plants not infected.

THE SOY BEAN BACTERIA.

Soy bean bacteria are evidently much less likely to be carried with the seed than are the cowpea bacteria. The soy bean plant grows more erectly than the cowpea (see Circular No. 69, "The Cowpea and Soy Bean in Illinois"), and the crop is quite commonly harvested with a self-

binder which keeps it quite free from dirt. The soy bean seed is nearly round and smooth, and the seed coat is not commonly cracked. These facts may explain why the soy bean seed carry so few bacteria as compared with cowpeas.

On one of the soil experiment fields on the university farm at Urbana, where soy beans have been grown for three years, no tubercles could be found on the plants either the first or second year, and only an occasional plant with tubercles could be found the third year. In 1902 a series of plots, some of which had been treated in different ways with applications of limestone, phosphorus, and potassium, were seeded with soy beans. No tubercles could be found at any time during the season on the soy beans growing on any of the different plots. In 1903 the same plots were again seeded to soy beans, and at the same time part of each plot was inoculated with infected soy bean soil drilled in with the seed at the rate of about 500 pounds of infected soil to the acre. When the plants were only a few weeks old tubercles were to be found upon many plants growing where the infected soil had been applied, and before the close of the season at least half of these plants in the inoculated part of the field had one or more tubercles upon their roots, and some plants could be found whose roots were abundantly provided with tubercles. (See Plate 4.)

On the uninoculated part of the field soy bean plants were examined probably fifty times during the season, several plants being taken up each time, but not a single tubercle was found at any time, notwithstanding that this was the second crop of soy beans upon this soil. Of course the inoculated part of the field did not become sufficiently infected to markedly benefit the 1903 crop, but it is planned to grow soy beans upon this field again in 1904 when the bacteria will doubtless have multiplied sufficiently to produce marked results in the growth of the crop.

From these and from other somewhat similar experiments it is concluded that as a rule soy beans should be inoculated when they are first seeded, and that they should then be grown a second year upon the same land. If soy beans are afterward grown upon this land once in every three or four years, the soil will doubtless remain well infected with the soy bean bacteria.

It is believed that 100 pounds of infected soy bean soil per acre will be sufficient to produce a thorough infection the second year, and it is improbable that one ton of infected soil per acre would produce a thorough inoculation the first season. One ton is only twenty times 100 pounds, while one tubercle which will be produced during a single season from a single bacterium may contain many million bacteria, thus it will be seen that it will be more economical to inoculate rather lightly and allow the bacteria to multiply themselves rather than to inoculate heavily at great expense.

PLATE 4. SOY BEAN ROOT TUBERCLES, NATURAL SIZE.

It may be stated that the infected soy bean soil used in these experiments was obtained from Mr. A. A. Hinkley of Du Bois, Illinois, who has been growing soy beans on the same land for many years until it has become well infected. Mr. Hinkley has consented to furnish infected soy bean soil so far as he is able to do without serious interference with his regular work, to any one who may desire it, at a price which will cover his expense and loss. This will probably amount to about $1.00 for the first 100 pounds and fifty cents for each additional 100 pounds, in the shipment, including the cost of bags, the purchaser to pay freight from Bois station, which is located in Washington County, Illinois, on the Illinois Central Railroad.

THE ALFALFA AND SWEET CLOVER BACTERIA.

That soil inoculation with alfalfa bacteria is commonly of very great value in growing alfalfa has been shown very conclusively by the investigations reported in Bulletin No. 76,* "Alfalfa on Illinois Soil." In some places, however, inoculation was found to be unnecessary. A careful and extensive investigation of alfalfa growing in different parts of Illinois revealed the fact, as stated in Bulletin 76, "that the alfalfa bacteria are certainly present in some places in the state while in most other places they are certainly not present in sufficient number to become of appreciable assistance to the alfalfa within three or four years, and the question naturally arises how it happens that some fields are already infected while others are not." It was suggested in that bulletin that the alfalfa bacteria may "live on some other plants besides alfalfa and that one of these plants is native or has been introduced in certain sections" of the state. It was also suggested "that a few bacteria are always carried with alfalfa seed, and that if the alfalfa is grown continuously or repeatedly in any place the soil will finally become thoroughly infected, and the bacteria will then be carried by flood waters, dust storms, etc., over adjoining fields, and possibly for long distances, especially along river valleys." This latter suggestion was known to be a fact at the time it was written; and subsequent investigations have furnished conclusive proof that the alfalfa bacteria do live upon another plant; namely, the ordinary sweet clover (*melilotus alba*). This is a rank-growing leguminous plant, frequently reaching a height of four to six feet. When young it markedly resembles alfalfa, but it can easily be distinguished by its characteristic odor when cut or bruised, as by rubbing between the hands.

*In this connection attention is called to the fact that the so-called "spot disease" of alfalfa, which is not uncommon in the western states, especially during wet seasons, became somewhat prevalent in Illinois in 1903. When the effect of this disease becomes marked, the leaves turn yellow and growth is retarded. If this occurs the alfalfa should be clipped. This is the only effective remedy known to be practicable. Seeding alfalfa with a light nurse crop is gaining favor in Illinois.

As the sweet clover approaches maturity it differs very much from alfalfa. The sweet clover grows very tall, and usually branches from a main stem. It has white flowers (there is also a less common yellow variety), and the seeds are borne in small round pods (usually containing only one or two seeds each), arranged on long slender spikes, each spike bearing many pods. The alfalfa commonly grows about two and a half feet high, with many stems growing from the crown of the root, especially after it is two or three years old. It bears purple flowers and peculiar spiral-shaped seed pods. Sweet clover is a biennial plant, dying after reaching maturity, which commonly occurs the second year of its growth. Like many other biennial plants, it probably often lives more than two years if not allowed to produce seed. Alfalfa is a perennial plant, and it is said that there are alfalfa fields which have been cut annually for more than fifty years without reseeding. The similarity of alfalfa and sweet clover when young, and also the similarity of the tubercles formed on the roots of each have long been noticed, and the possibility of the same bacteria living upon both plants has already been suggested in the agricultural press.

During the season of 1903 the writer spent some time in the northern part of Illinois in connection with the general and detail surveys of Illinois soil. Many new fields of alfalfa were observed, and they were carefully examined for root tubercles. In Winnebago County, where sweet clover is very prevalent along roadsides and in waste places, it was noted that the abundance of root tubercles on the alfalfa plants seemed to be closely related to the presence of sweet clover in the vicinity, strongly indicating that the bacteria which live upon sweet clover were also at home upon the alfalfa roots. These indications were strengthened by further investigations in Lake County, especially upon the Fowler farm, near Lake Villa, where a field of alfalfa seeded last spring without artificial inoculation was found to be thoroughly infected with the bacteria, and growing vigorously with a good dark green color. This field had a few sweet clover plants growing in it, and the borders of the field were covered with sweet clover. Other fields of alfalfa seeded in the neighborhood at the same time, but upon soils where sweet clover had not grown near by, were apparently complete failures, many of the plants having died and most of those still living being only a few inches high, very weak, and yellow or pale green in color.*

In order to obtain more absolute knowledge regarding this important subject, a series of pot culture experiments has been carried on under controlled conditions in the pot culture laboratory at the university. Five pots were filled with sterilized sand which was practically devoid

*Some of these observations have already been reported in the agricultural press. (See, for example, the Breeders' Gazette, September 9, 1903, page 391, and September 16, 1903, page 442.)

of plant food. A supply of phosphorus, potassium, and all other mineral elements necessary for the growth of plants was added to each of the five pots, care being taken to keep the sand practically free of combined nitrogen. Alfalfa seed were then planted in each of the five pots, and at the same time four of the five pots were inoculated as follows:

Pot No. 1.—Not inoculated (check pot).

Pot No. 2.—Inoculated with bacteria obtained from infected alfalfa soil.

Pot No. 3.—Inoculated with bacteria obtained from alfalfa root tubercles.

Pot No. 4.—Inoculated with bacteria obtained from infected sweet clover soil.

Pot No. 5.—Inoculated with bacteria obtained from sweet clover root tubercles.

Plate 5 clearly shows the results obtained and certainly furnishes conclusive proof that the same effect is produced upon the growth of the alfalfa whether the nitrogen-gathering bacteria used for the inoculation are obtained from alfalfa soil, from alfalfa tubercles, from sweet clover soil, or from sweet clover tubercles. It also illustrates the importance of bacteria in growing alfalfa as will be seen by comparing the four inoculated pots with the uninoculated pot, which is No. 1, on the left in each series of views. The upper view was taken when the alfalfa plants were five weeks old; the next series when they were six weeks old; the next, seven weeks old; and the lower series when they were eight weeks old, from the time of seeding.

A duplicate series of pots prepared in exactly the same manner gave similar results.

The infected alfalfa soil was obtained from a field of three-year-old alfalfa which was inoculated when first seeded, with infected alfalfa soil obtained from an old alfalfa field in Kansas. About one pound of this soil was shaken in a quart of water, the soil allowed to settle, and some of the nearly clear solution used for the inoculation of Pot No. 2. The alfalfa tubercles from which bacteria were obtained were carefully washed in distilled water to free them from adhering soil particles, and then rubbed up in distilled water, a small amount of this water being then used for the inoculation of Pot No. 3. The infected sweet clover soil was obtained from a place by the roadside where sweet clover was growing luxuriantly and well provided with root tubercles. This place was about two miles from the nearest field ever seeded to alfalfa, so far as known. A water extract from this soil was used to inoculate Pot No. 4. The bacteria from sweet clover tubercles were obtained in the same manner as those from alfalfa tubercles, and were used to inoculate Pot No. 5.

From these investigations we thus have conclusive evidence that infected sweet clover soil can be used for the inoculation of alfalfa fields,

PLATE 5. ALFALFA: EFFECT OF BACTERIA FROM ALFALFA AND FROM SWEET CLOVER.

Pot 1.—No bacteria.
Pots 2 and 3.—Bacteria from alfalfa.
Pots 4 and 5.—Bacteria from sweet clover.
The four series of photographs were taken five, six, seven, and eight weeks from time of planting, respectively.

the bacteria of the two plants acting the same. The infected soil may be obtained from any place where the sweet clover is found growing with abundance of tubercles on its roots. The soil may be collected to a depth of three or four inches and scattered over the alfalfa field at the rate of 100 pounds or more to the acre. It is well to scatter the infected soil at about the time the alfalfa is seeded, and harrow it in with the alfalfa seed, although it may be applied some days or even some weeks

before seeding time, and probably it would be all right to apply the infected soil the fall before, for it is known that the bacteria will live in soil for several months, even though the soil be placed in sacks and allowed to become quite dry.

Investigations have shown that 100 pounds of thoroughly infected soil to the acre is sufficient to produce a very satisfactory inoculation within one year from the time it is applied. Of course, somewhat heavier applications may well be made if it can be done at small expense. The infected soil need not be applied with any high degree of uniformity, but special care should be taken that the higher places and watersheds are not missed in scattering it over the field. If a few square yards, or even square rods, should be missed on the slopes or lower land, it would make but little difference, as the bacteria will be washed over such places from the higher land.

After the soil becomes somewhat dry it is easily scattered by hand from the wagon or from a sack which one can carry. Sometimes it is applied by means of an end gate seeder or a fertilizer drill, or it could be spread by a manure spreader with an application of manure.

The question naturally arises whether there is not danger of getting some sweet clover seed with the infected sweet clover soil, and thus of getting sweet clover mixed with the alfalfa in the field.

In the writer's opinion there is little or nothing to fear in this matter. In the first place, the amount of sweet clover seed thus obtained would be very small, probably none at all, if one were careful to scrape off the vegetable matter, and perhaps a half inch of earth before collecting the infected soil (most of the bacteria are probably between one-half inch and six inches in depth, as most of the tubercles develop and decay between these depths); second, it is doubtful if a small amount of sweet clover hay would lessen the value of alfalfa hay in the least, for stock frequently eat small amounts of sweet clover of their own choice even when it is nearly mature, and if it is cut while still quite immature and tender it makes quite satisfactory hay, so much so that in some sections of the United States, particularly in the South, sweet clover is regularly seeded on fields and cut for hay, and it is found to be a valuable and very nutritious feed, the live stock eating it in large quantities, and with apparent relish, after they have acquired a taste for it; third, sweet clover is not known as a bad weed in the fields or meadows, even where it has been a common roadside plant for many years, and, being naturally a biennial plant, if it were cut down every five or six weeks, as we commonly cut alfalfa during the season, it would almost certainly die out after a few years while alfalfa, a perennial plant, would continue to live.

Only one instance has come to the writer's attention where alfalfa has been growing for several years with sweet clover growing in the

field or fence rows beside it. This is on the farm of Mr. D. S. Mayhew, of Mercer County, Illinois, who writes as follows regarding the matter:

"Will say that the sweet clover has made no headway in my meadow, as it did not go to seed, on account of my cutting it so often. The sweet clover got into the alfalfa in the seed when I sowed it. I do not think it will do any harm in a meadow, but I believe it would do harm in a pasture if it wasn't cut down' as stock will not eat the sweet clover."

Of course if sweet clover should get into the field and persist in growing, and if it were found to injure the alfalfa appreciably or markedly, we can always resort to plowing the ground up and growing corn or other crops, thus obtaining some benefit from the leguminous crop for its fertilizing value, and at the same time completely eradicating the sweet clover, but leaving the soil well infected with alfalfa bacteria ready to serve in case alfalfa should be again seeded within a few years.

CONCLUSIONS.

In general agriculture in Illinois, whether it be grain farming or ordinary livestock farming, the growing of legumes is absolutely essential as a part of any economic system which shall maintain the fertility of the soil; and for the successful growing of legumes the presence and assistance of the proper species of nitrogen-gathering bacteria are also absolutely essential. These facts being granted, it certainly follows that when sowing any legume on land, where the same legume has never been grown before, or perhaps where it has not been successfully grown within recent years, we should always consider the matter of inoculation; and, unless there is good reason to believe that the soil has been inoculated by the washing from other higher lying land where these bacteria are known to be present or by applications of manure made from that legume, or by some other such incidental means; or unless there is evidence that the bacteria are carried with the seed in sufficient quantity to effect a satisfactory inoculation (as appears to be the case with the cowpea), then we should inoculate the soil directly with the specific bacteria required by the legume which we desire to grow.

While some Illinois soils are becoming deficient in phosphorus and in lime, especially in the southern part of the state, and while phosphorus* and ground limestone can be applied to such soils with marked benefit and profit, especially for the growing of legumes, there is abundant evidence that one of the dominant causes for the failure or unsatisfactory growth of some of our most valuable legumes, and on some soils the sole cause of failure, is the absence of the proper nitrogen-gathering bacteria.

There is no reason to believe that any of the different species of nitrogen-gathering bacteria will live in the soil for more than a few

* Steamed bone meal is the most economical and satisfactory form of phosphorus for use on Illinois soils, unless ground rock phosphate (not acid phosphate) shall prove to be still more economical. Experiments to determine this are in progress.

years* in the entire absence of any legume upon which they naturally live, and the accumulating evidence strongly indicates that the bacteria which are present in places in our soils, such as the red clover bacteria, now found abundantly in many places in the state, especially in northern and central Illinois, the cowpea bacteria more common in southern Illinois, and the alfalfa or sweet clover bacteria, which are becoming prevalent in some sections—that all these have been, and are being, gradually introduced and extended almost entirely by mere chance. Of course if the wagon-wheel, which carries the mud along the road, carries with it sweet clover seed from one place to another, it may also carry the sweet clover bacteria which live on the sweet clover roots.

It now seems absurd to suppose that there were red clover bacteria in Illinois soil before red clover itself was grown on Illinois soil, unless the same bacteria live also upon some other legume which was native to our soils. There is some evidence that the vetch bacteria are native to our soil, possibly living upon the native wild vetches. At any rate, tubercles commonly develop on vetch roots without artificial inoculation. Investigations are in progress to ascertain whether the notorious failure of crimson clover in Illinois may not be due in part, at least, to the absence of the proper bacteria. (It has been stated by some writers that the bacteria of crimson clover and those of red clover are identical, but we already have some reason to doubt the accuracy of this statement.)

*Just how long the bacteria will live in a soil without a leguminous crop upon which they can feed is not definitely known. Certainly they live for two or three years, but probably not more than five or six years. Further investigation is needed to establish the length of time the different kinds of bacteria may remain in the soil under different conditions.